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BRIEF COMMUNICATION

Long-Term Food Restriction, Deprenyl, and Nimodipine Treatment on Life Expectancy and Blood Pressure of Stroke-Prone Rats

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STEVENS, S., S. KNOLLEMA, G. I. DE JONG, J. KORF, AND P. G. M. LUITEN. *Long-term food restriction, deprenyl, and nimodipine treatment on life expectancy and blood pressure of stroke prone rats.* NEUROBIOL AGING **19**(3) 273–276, 1998.—We determined whether food restriction or the drugs nimodipine (Ca^{2+} antagonist) and deprenyl (a MAO-B inhibitor) prevent the development of stroke in the spontaneously hypertensive stroke-prone rat (SHR-SP). Forty male SHR-SP rats, in the age of 34 weeks, were exposed to various treatments. During a period of 27 weeks, survival and blood pressure were followed. In the control and deprenyl group, the blood pressure values remained unchanged; 50% had died after 27 weeks. All rats that were treated with nimodipine survived. After food restriction, 7/8 rats survived and showed a lower blood pressure. This study in SHR-PR rats shows the superiority of nimodipine on survival, and the potential of food restriction as a stroke-preventing measure. © 1998 Elsevier Science Inc.

Stroke prevention Deprenyl Nimodipine Food restriction Stroke-prone rats Hypertension Blood pressure

ESSENTIAL hypertension is a major risk factor for stroke (21). The spontaneously hypertensive stroke-prone (SHR-SP) rat strain (20) may serve as a model of hypertension and stroke. At approximately the age of 43–52 weeks, SHR-SP rats develop cerebral hemorrhages and infarcts located in the boundaries of the flow region of the middle cerebral artery. As a consequence, the lifespan of SHR-SP rats is considerably shortened, which makes SHR-SP rats particularly suitable to assess prophylactic interventions. Of the cytotoxic events leading to neuronal death following cerebral ischemia, excessive calcium influx, acidosis, and free radicals are crucial (3,8,9,12,17,22–24,). Previously, it was demonstrated that long-term treatment with the calcium antagonist nimodipine prevented neocortical strokes in SHR-SP rats up to 56 weeks (12,13). The beneficial effects of treatment with free radical scavengers in acute or global cerebral ischemia models are suggested in several studies (1,8). Free radicals are formed by monamine oxidase type B (MAO-B), which may explain not only the neuroprotection in stroke models (e.g., 8), but also the longevity of (normotensive) rodents after long-term treatment with deprenyl (2,6,11). Another way to protect against stroke is by starvation, which possibly is caused by a diminished hyperglycaemic response after hypoxia (3,15). Interestingly, normotensive rodents extend the lifespan following food restriction.

Here, we describe the efficacy of long-term food restriction and treatment with deprenyl or nimodipine on blood pressure and survival of adult SHR-RP rats.

MATERIALS AND METHODS

Animals and Treatment

Forty male SHR-SP (breeder Møllegaard Scønevød, Denmark) were housed in groups of 6 or 4 animals. At the age of 34 weeks animals were divided into 5 groups and individually housed. Accordingly, the 1) Onset-control group ($n = 4$); 2) Control group ($n = 12$) and 3) Deprenyl group ($n = 10$) had free access to water and standard lab chow. An oral daily dose L-deprenyl 5 mg/kg was added to the drinking water and adjusted weekly to maintain the dose within 90–111% (1,2). The *Food restriction group* ($n = 8$) received 60% of the normal food intake with Vitamin supplements. The volume of this food was normalized to the volume of food in the control group with ingestible cellulose. To the *Nimodipine group* ($n = 6$) water and food were supplied ad libitum, while the

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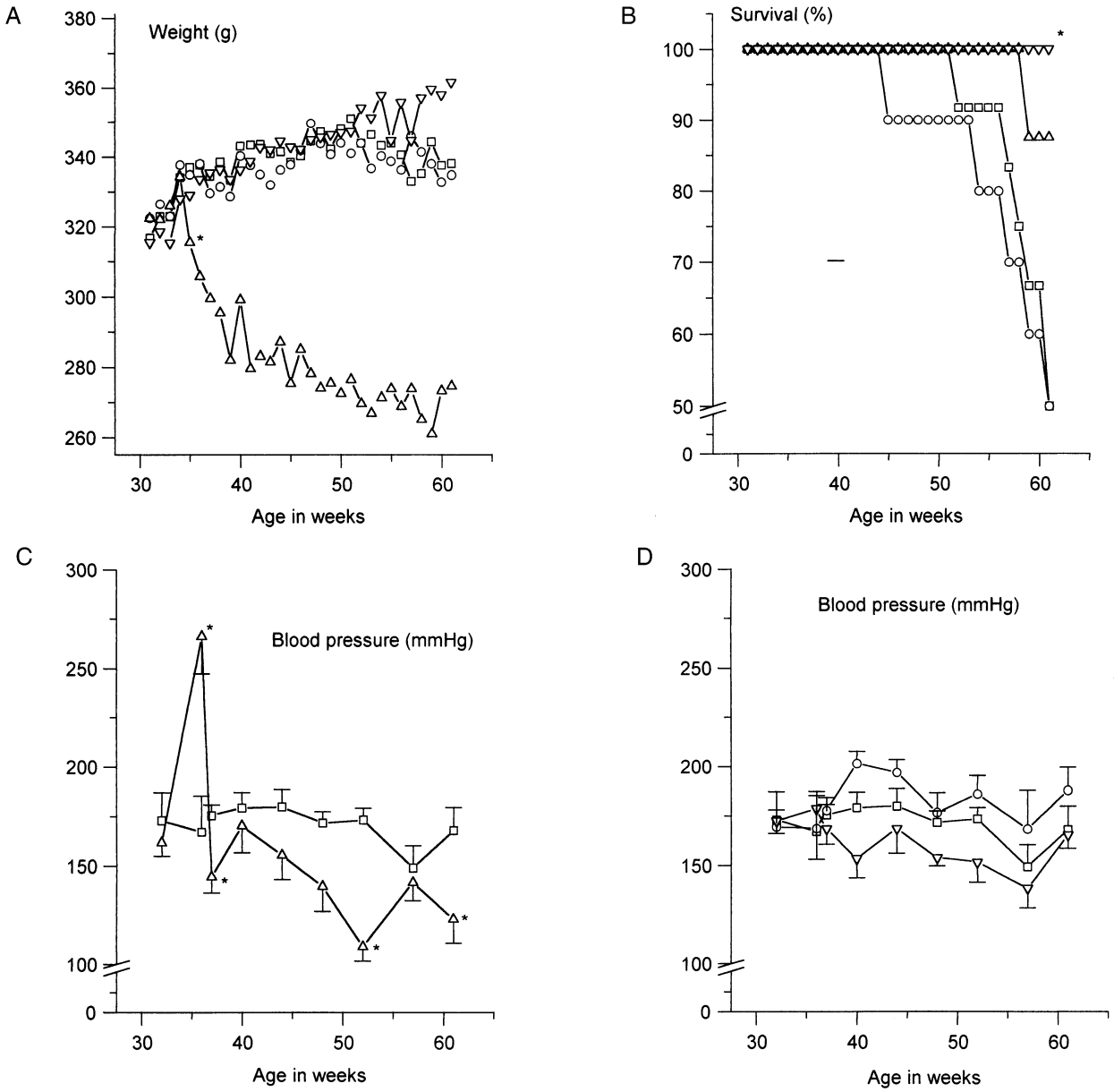

FIG. 1. Changes in bodyweight, survival, and blood pressure during various treatment regimens of adult SHR-SP rats. Rats were either given nimodipine, deprenyl, or a low amount of food. Results are given as mean ± SEM, during the treatment period. In A, * indicates time point from where the weight in the food restriction group differs significantly ($p < 0.05$) from controls or any other group. In panel B the survival rate following various treatments differed significantly from control animals at * ($p < 0.05$). In C and D the blood pressure after food restriction increased transiently and decreased significanty thereafter (* $p < 0.05$) as compared to control or other treatment groups. -□-: controls; -○-: deprenyl; -△-: food restriction; -▽-: nimodipine. Number of observations per Group 6–10 rats.

latter contained 860 ppm nimodipine (Bayer AG, Leverkusen, Germany). The drug treatments and food deprivation regimen were carried out for 27 weeks (from 34–61 weeks) and the body weight was recorded weekly. At ages of 32, 36, 37, 40, 44, 48, 52, 57, and 61 weeks systolic blood pressure (SBP) was measured. The SBP measurement was carried out under light ether anesthesia with the tail-cuff method using a photoelectric sensor unit to detect arterial pulse visualized on an oscilloscope. The pressure in the cuff placed around the base of the tail was gradually elevated until the pulsation disappeared. During gradual decreasing of the pressure the SBP was read on a manometer at the moment of the reappearance of pulses, expressed in mmHg.

The animals were checked daily on hyperirritability, hemiplegia, apathy, and motor disturbance. Brain weight (excluding olfactory bulb and lower medulla) and appearance of stroke and brain edema was assessed in the Onset-control group after 34 weeks, and at the age 61 weeks for the surviving animals. Cell damage in the surviving animals was visualized with silverstaining (25). Blood glucose levels were measured at 32 weeks and 14 days after the start of the treatments. Blood samples for glucose

measurements were obtained in the first half of the light period. Blood samples were immediately transferred to a chilled (0°C) medium containing EDTA and 10 μL heparin (500 U/mL). Blood glucose (50 μL blood) was measured by the ferricyanide method of Hoffman (Technicon Auto Analyzer TMII). The protocol was approved by the committee supervising animal experimentation of the Faculty of Medical Science (University of Groningen).

Data Analysis

Investigators were blind to the procedures during blood pressure measurements, weighing, glucose analysis, and histology. Data shown are presented as mean ± SEM. Blood pressure value and weight each different time points were statistically evaluated using one-way analysis of variance (ANOVA), which in case of significant group differences, was followed by a post hoc Bonferroni test. To establish the effect of the different treatment strategies in time and the interaction of time and treatment on blood pressure we used a RM-ANOVA evaluated by a F-test. In this test time and treatment are a factor with a first-order interaction between time and treatment. Survival data were statistically evaluated using Kaplan-Meier survival analysis, and to test the equality of the survival distributions for the different groups we used the Log-Rank test. Differences were defined as significant when $p < 0.05$.

RESULTS

General Condition

The bodyweight of the SHR-SP rats during treatment with nimodipine and deprenyl was not significantly different from that of controls, whereas the bodyweight of food restricted rats was from Week 36 onwards significantly lower (until 81% of the bodyweight at 32 weeks; Fig. 1A). The blood glucose levels (in mM/L ± SEM, 4–10 observations) as measured in all 4 groups at the age of 32 weeks and 14 days after onset of the treatment were 7.85 ± 0.40 (control), vs. 7.71 ± 0.57 (food restriction), 7.93 ± 0.51 (deprenyl), and 7.63 ± 0.67 (nimodipine). No significant differences were found between the groups. Of the surviving rats at the age of 61 weeks the brains did not reveal signs of infarction or edema (data not shown).

Survival

None of the nimodipine-treated animals died and their survival rate was significantly increased ($p < 0.05$). Of the control, deprenyl-treated, and food-restricted rats, 50%, 50%, and 12.5%, respectively, died. All the animals that had died before the end of the experiment displayed neurological symptoms of stroke and most likely died as a consequence of stroke. Figure 1B shows the survival rate in the different groups during the experiment.

Blood Pressure

Figures 1C and D show the time course of blood pressure. Food-restricted rats showed at the age of 36 weeks a temporary but significant ($p < 0.05$) rise in blood pressure (266.0 mmHg ± 18.0 vs. 167.2 mmHg ± 18.7, in controls) (Fig. 1c) and a significantly ($p < 0.001$) lowering effect thereafter. Long-term treatment with deprenyl or nimodipine did not affect blood pressure (Fig. 1D).

DISCUSSION

We confirmed and extended the observation (12) that nimodipine treatment prevents stroke and enhances survival of SHR-SP rats. New are the data that long-term deprenyl treatment does not prevent stroke nor influence the survival rate, and that food restriction may offer neuroprotection in SHR-SR rats. MAO inhibitors are known to give a rise in blood pressure, but deprenyl is an exception (5). The present data are in line with the clinical observation (6). A dose of 5 mg/kg p.o. was used, which corresponds to the ED_{50} for the selective cerebral MAO-B inhibitor after oral administration in the rat (1). Apart from the neuroprotective effect in cerebral ischemia, deprenyl treatment is also claimed to increase the life-expectancy in rodents and patients suffering from Parkinson disease (6,7). However, conflicting data are published concerning the safety of long-term deprenyl use (e.g., when combined with Levodopa; (10)).

Food restriction is generally known to increase life expectancy and to retard the occurrence of age-associated processes such as nephropathy, cardiomyopathy, and neoplasia (14,16,28). The present study shows the prophylactic effect of food restriction on the development of hypertension when food restriction is started at adult age. To our knowledge, the present study is the first to describe the effect of food restriction on lifespan and blood pressure in SHR-SP rats when given at adult age. Other studies showed a decrease in blood pressure or/and an increase in lifespan after food restriction in hypertensive animals if food restriction was performed for a short period (4 days) or for a longer period up to 8 weeks only in young animals (4,5,18,19,26,27). The lowering effect of food restriction on blood pressure did not result in a significant increase in survival in our study, although this may be largely due to the small sample size. The initial rise in systolic blood pressure in the first 2 weeks in this study may be the result of a stress effect of food deprivation. Stroke in the SHR-SP rats is hypothesized to be the result of an enhanced vulnerability of the vasculature for pressure load caused by an altered vascular metabolism and physical damage to the vessel wall from the high blood pressure (20). To what extent the changes in metabolism may play a role in the increased survival is not clear from the present study, because blood glucose levels are measured only at one time. However, our results indicate that changes in blood pressure may be independent of the blood glucose levels, because these levels do not change after the start of food restriction.

In conclusion, this preliminary study shows that nimodipine prevented stroke most effectively, that food restriction had a profound beneficial impact on hypertension by decreasing blood pressure in SHR-SP rats, and that the results with deprenyl were negative. Both the effective treatments either given apart or in combination may provide neuroprotection in stroke-prone individuals even when started in the adult.

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